BIOTECHNOLOGICAL METHODS REDUCING THE METHANE PRODUCTION IN RUMEN

BIOTEHNOLOŠKE METODE ZA SMANJENJE PROIZVODNJE METANA U BURAGU

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SUMMARY

While methane, produced as a result of fermentative digestion in the rumen of ruminants and spread into the atmosphere, mostly causes the loss of energy received by feed for animals, it also has a negative effect on the global warming. Biotechnological methods aimed at limiting methane production, which constitutes ecological problems in terms of oscillation into the environment and economic problems in terms of feeding animals, are the methods such as; improving the nutritional value of feedstuff by using biotechnological additives and graft/vaccine and transgenic organisms. These methods have found an extensive area of usage especially in the recent years. In this review paper, the research such as formation of ruminal methane, bacteria which produce methane (methanogen) and biotechnological methods reducing methane production in the rumen have been included. When assessing the available research, it is concluded that biotechnological products and applications used as feed supplement reduce methane production as a result of suppression of the usage of hydrogen of methane producing bacteria, by increasing propionic acid production in the rumen.

Key words: methane production, methanogenesis, rumen fermentation, biotechnological methods

INTRODUCTION

Ruminants are capable of the digestion of structural carbohydrate ingredients (cellulose, hemicelluloses) by means of microorganisms located in the gastrointestinal tract and entering into a symbiotic relation with the animal. Thus these structural carbohydrates are converted to nutritional forms like meat or milk which the human could utilize. However methanogenesis (methane production) that is formed as a result of digestion in the rumen has negative aspects such as inadequate digestion of the foodstuff and loss of nitrogen. The methane (CH4), produced by the mathanogenic bacteria as a product of the anaerobic carbohydrate metabolism, causes not only the loss of 10% of energy in the animals but also adverse effects on the global warming (Meral ve Biricik 2013, Öztürk H. 2007, Güçlü and Kara 2010).

The yearly methane production of the adult ruminants globally is approximately 80 million tones (Fonty and Morvan 1996). Researchers report that the methane in the atmosphere causes 10-18% of the global warming and 15-20% of this methane is generated by bigger ruminants (Scheehle and Kruger 2006, Steinfeld et al. 2006). Ruminants, contribute directly to the methane aggregation by the methane they generate as a result of fermentative digestion, and indirectly by anaerobic disintegration of the stool. In animals fed with rations of poor quality, as a result of inadequacy of the microbial nutritional source, the microbial growth is also inadequate and methane production may rise up to 75% (Iqbal et al. 2008). The energy lost with methane constitutes a problem both economically and ecologically. Because methane is, apart from leading to energy loss due to rumen fermentation, a green-

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house gas causing global heating. It is reported that its contribution in the generation of anthropogenic greenhouse effect after carbon dioxide is 18%. The role of ruminants in anthropogenic greenhouse effect takes its source from this (Moss et al. 2000). In the rumen of an adult cattle, 500-1500 liters of gas is generated a day and this gas constitutes 50-60% of CO_2 and 30-40% of CH_4 (Öztürk 2007). For this reason, strategies reducing methane diffusion take primacy. These strategies are important in short term for enhancing the animal performance and avoiding energy loss, and in long term for environmental protection. Withholding the hydrogen ions produced in the rumen from utilization of methanogenic bacteria will cause significant economic and ecological gains. Various methods are applied for this purpose. In this review paper, the applied biotechnological methods to reduce methane formation in the rumen are elaborated.

BACTERIA WHICH GENERATE METHANE (METHANOGENS)

The methanogenic bacteria transform H2 and CO2 to methane. The methanogens are generally found at high levels in the rumen's microbial ecosystem (10^8 - 10^9 cell/ml) (Fonty and Morvan 1996, Kamra 2005, Kumar et al. 2009). Methanogens live in strong anaerobic conditions and by reducing hydrogen with carbon dioxide to produce methane; they procure their whole metabolic energy. There is a symbiotic relation between the mathanogenic bacteria and protozoa with cilia (Ohene-Adjei et al. 2007). Methanogens are classified as arkea and divided into five significant groups such as; *Methanobacteriales, Methanosarcinales, Methano-coccales, Methanomicrobiales, and Methanopyrales*. Methanobacteriales are dominant in the rumen. Methanogens such as *Methanobrevibacter ruminantium, Methanomicrobium mobile and Methanosarcina* are the most important methanogens in the rumen microbial ecosystem, isolated in the sheep and cattle rumen. It is reported that the total methanogens in the rumen form 3.6% of the microorganisms in the rumen and 54% of this rate is *M. mobile* (Yanagita et al. 2000).

RUMINIAL METHANE GENERATION (METHANOGENESIS)

Methane production in the rumen changes depending on various factors such as; energy content of the feed, its quality and quantity, the type and size of carbohydrates fermented in the reticulorument, the rate of propionic acid production to the rate of acetic acid, the weight and age of the animal, differences between the types of animals and individualism in the same species (Moss et al. 2000, Ungerfeld et al. 2004, Nkrumah et al. 2006, Kılıç and Şimşek 2009). The methane gas formed in the rumen generally comes out as a result of microbial fermentation of the hydrolyzed carbon hydrate such as; cellulose, hemicellulose, pectin and starch (Kebreab et al. 2006). Apart from this, it was observed that substantial methane was produced in the rumen of the animals fed high protein rations due to fermentation (Mills et al. 2001). In the rumen, where microbial digestion takes place at peak intensity, the microorganisms fermentatively digest the feed the animal eats, so they breed and increase in number. As the product of the fermentation they create volatile fatty acids (VFA), the most important of whom are known as acetic acid, propionic acid, and butyric acid. VFAs, absorbed into the blood through rumen epithelium, meet 75% of the energy need (Fonty and Morvan 1996, Faverdin 1999, Öztürk et al. 2001, Ünay et al. 2008). Amongst the latest products of the microbial digestion, apart from volatile fatty acids, there are hydrogen ions and CO_2 (Demeyer et al. 1996). The released hydrogen is transformed into CH_4 by mathanogenic bacteria with $4(2H) + CO_2 \rightarrow CH_4 + 2H_2O$ reaction (Johnson and Johnson 1995, Demeyer et al. 1996). The microorganisms which breed and increase in the rumen lead to abomasums and small intestine and then they are decomposed by digestion enzymes and become protein and vitamin source for the animal (Varga and Kolver, 1997, Öztürk 2008, Sarı et al. 2008). Moreover, approximately 12% of the brute energy taken with feed is transformed to methane gas during the microbial digestion in the rumen, and it is released to the atmosphere by eructation; for this reason, formation of CH_4 gas means unproductive usage of feed energy (Johnson and Johnson 1995, Meral ve Biricik 2013).

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BIOTECHNOLOGICAL FEED ADDITIVES EFFECTIVE IN DECREASING THE METHANE IN THE RUMEN

The characteristics of the ration the animal eats directly affect the efficiency of the animal and rumen metabolism. In such studies, probiotics of biotechnological products and organic acids are frequently mentioned.

Probiotics: They are the main products of alternative biotechnological products (Karademir and Karademir 2003). While probiotics are used to enable the transformation of methane into carbon dioxide in the ruminants and avoid energy loss, it is stated that its effect on the global warming is also reduced (Sarıpınar and Sulu 2005). By means of probiotic administration, the level of propionic acid in the ambient is increased and as a result of reducing the hydrogen (pre substance of methane) and formic acid, 4 - 31% reduction in the amount of methane produced in the rumen is ensured The fungal probiotics used for increasing efficiency in the adult ruminants, remove elements such as sugar, toxic metals and oxygen by way of increasing the growth of fungus such as *Neosallimistic frontalis* in the rumen, and they display a low rumen pH by way of increasing the production of probionic acid with the number of cellulolytic bacteria living in the rumen and the number of bacteria using lactic acid (Benchaar et al. 2001, Lila et al. 2004).

Prebiotics: The increase in cellulolytic rumen bacteria is provided by using the compounds such as mannan - oligosaccharide (MOS) specified as prebiotic, fructo – oligosaccharide (FOS), alfa - galacto - oligosaccharide (α-GOS) galactocile - lactose, inuline, enzymatically hydrolyzed inuline (oligofructose) and synthetic fructose, which means the animal obtains more net energy for the efficiency ratio it takes from the feed (Sarıpınar and Sulu 2005, Güçlü and Kara 2010).

Organic acids: It is reported that in order to reduce the methane production by bacteria in the rumen and convert the energy to the compounds which the animal can use, methanogenesis can be reduced by restricting the formic acid production and hydrogen forming the methane by way of increasing the propionic acid production and the level of decomposition of structural carbohydrate (cellulose, hemi cellulose, lignin) of the feed in the rumen (Asanuma et al. 1999, Khampa and Wanapat 2007). In recent years, it has been reported that adding malic acid, fumaric acid and salts in the rumen rations, or by the studies carried out in vivo and in vitro with these organic acids, depending on reducing the acetic/ propionic acid within the total VFAs and increasing the propionic acid, has a reducing impact of the production on CH_4 in the rumen (Asanuma et al. 1999, Martin et al. 2000, Khampa and Wanapat 2007, Foley et al. 2009). It is reported that malic acid is more effective when increasing the ability of benefiting from lactic acid as carbon and energy and the fumaric acid is more effective when providing energy close to the level of glucose as metabolized, thus by preventing methanogenesis it avoids energy loss (Martin 1998). Castillo et al. (2004) reported that by adding fumarate into the feed, the bacteria using fumarate fight against the bacteria producing methane for H_2 and thus become effective in reducing methane production. Odongo et al. (2007) reported that the addition of myristic acid (C 14:0) into the rations of the dairy cattle increases methane production up to 36%. Mc Ginn et al. (2004) reported that addition of the yeast and fumaric acid into the feed did not have any impact on the methane production. It was brought forward by Itabashi vd (2000) that in Holstein cattle, which were fed rations with fumaric acid supplement and salinomycin, methane was reduced by 16% based on the increase of propionate in the rumen. The same researchers reported that the addition of fumaric acid into sorghum silage enabled 23% reduction in methane emission.

Plant extracts: Active compounds in the aromatic plants (e.g carvacrol, thymol, eugenol, cinnamaldehit) affect the microbial activity in the rumen due to their natural productive and anti-microbial ethics (Wallace et al. 2004, Hart et al. 2007, Benchaar et al. 2008). The plants and the volatile oil acquired from them are the secondary compounds of the plants, and due to their anti-bacterial, anti-fungal, and anti-oxidant characteristics, they display antimicrobial activity against the gram positive and gram negative bacteria (Castillejos et al. 2005, Hart et al. 2007). It is reported that some volatile oils make changes on the rumen volatile oil acids, and reduce the speed of amino acid deamination, ammonia production speed and number of bacteria producing ammonia at a considerable rate (Evans and Martin 2000, Wallace et al. 2002, McIntosh et al. 2003). For this reason, natural plant extracts can be used for the selective arrangement of specific microbial species. With the usage of plant extracts enjoying antimicrobial activity, it is possible to reduce rumen microbial activity (Cardozo et al. 2004).

IMMUNIZATION

One of the strategies of reducing methane emission generating from ruminants is reducing the methane release in the animals which acquire immunity to population of methanogens and protozoa they contain in their rumen (Ulyatt and Lassey 2001, Iqbal et al. 2008, Mitsumori and Sun 2008). Gill et al. (2000) ve Shu et al. (2000) stated that the vaccine developed against the species such as Streptococcus bovis and Laktobacillus spp., causing lactic acidosis in the rumen, made an immune response to the protozoa due to the presence of the specific antibody in the contents of the rumen and this antiprotozoal impact directly affected the activities of the methanogens, which were in close relation with the protozoa. Wright vd 2004 reported that the effects of the developed vaccine, notably VF3 based on 3 methanogen strains and VF7 based on 7 methanogen strains as antigen was researched and 4 months after the vaccine was administered, the formation of CH4 reduced by 6%, which was statistically insignificant. However, upon repeating the administration, at the rate of 7.7% reduction was reported for the methane emission as per kg dry matter and this rate was found statistically significant. In a similar research, it was reported that as a result of the administration of a vaccine based on 5 methanogen strains on the sheep, 18% emission reducing impact was observed (Williams et al. 2009). Moreover, it was reported that the structure of the ration used and physiologic conditions in the rumen were effective for the high impact of the administration of vaccine to methanogen (Wright et al. 2007). It was reported by some researchers that the *Methanobrevibacter ruminantium M1* fractions had been used recently and they were in a process of developing an antimethanogenic vaccine, whose impact was not known yet, and this could reduce the CH_4 up to 70% (Wedlock et al. 2010).

BACTERIOPHAGES

The bacteriophages, found in the rumen microbial ecosystem at the rates of 10^8 - 10^9 cell/ml, are defined as viruses, whose eukaryote cells are infected (Kamra 2005, Buddle et al. 2011). Bacteriophages, found abundantly in the rumen with genetic variability, enable gene transfer from the aerobic and facultative anaerobic microorganisms. They bind to the specific receptor on the surface of the bacteria in order to get into the host cell. Then the host, which they transfer their genes convert into a phage similar to their characteristics (Brussow et al. 2004, Stanton 2007, Chen and Novick 2009). It is reported that there are two methanogen genes (Mathanobacterium phage M1-M2 and Methanothermobacter phage M100) carrying the receptors that bacteriophages can bind to and be produced with the help of in vitro techniques (Buddle et al. 2011). Attword and McSweeney (2008) reported that as the lytic enzyme gene, which has a characteristic of eliminating harmful microorganism species, is able to hold on to the cell proteins of the *M. ruminantium,* it may achieve a phage with a biocontrol mechanism reducing the methane emission in the rumen as a result of its transfer to the *M. ruminantium.*

RECOMBINANT - DNA TECHNOLOGY

Recombinant DNA technology has become a highly strong modern approach for the enhancement of the activity of the ligno - cellulolytic enzymes. With this technology, new gene products can be achieved by changing the characteristics of lingo - cellulolytic genes of microorganisms (Çömlekçioğlu et al. 2011). With this application, where the rumen microorganisms can be modified by rDNA technology; it is reported that methane production can be reduced with the increase in cellulolytic activity parallel to the decomposition of ligno - hemi-cellulose complex (Türkmen et al. 2011). Sar et al. 2005; reported that in the rumen ambience where E. *coli* W3110 and E. *coli* nir-Ptac anaerobic cultures coded with nitrit reductase are applied, methane production considerably reduces.

CONCLUSION

Today, the biotechnological development contributes much to the efforts to increase the amount and quality of the productivity, accordingly the profit, which is the main objective of the livestock farming. Organic acids and probiotics included in biotechnological products, put in the service of the animal breeders, are among the most appropriate feed additives due to being natural and safe for human health. On the other hand, conditions in the rumen make the gene transfer possible in the same or different microorganism groups. Regarding the administrations devoted to methane reducing strategies, which have both ecological and economic importance, apart from the usage of available biotechnological additives, more research should be made on the transgenic microorganism and vaccine techniques, and at the same time their utilization should be popularized.

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SAŽETAK

Metan, koji nastaje kao posljedica fermentacije probave u buragu preživača i širi se u atmosferu,većinom uzrokuje gubitak energije koju životinje dobivaju hranom te isto tako negativno djeluje na opće zatopljenje. Biotehnološke metode radi ograničavanja proizvodnje metana koji stvara ekološki problem u smislu oscilacije u okolinu i ekonomski problem u smislu hranidbe životinja su poboljšanje nutritivne vrijednosti hrane primjenom biotehnoloških dodataka, inokulanata i transgenih organizama. Te su metode naišle na široko područje primjene, osobito zadnjih godina. Ovaj članak uključuje istraživanje stvaranja metana u buragu, bakterija koje proizvode metan (metanogen) i biotehnoloških metoda koje smanjuju proizvodnju metana u buragu. U ocjenjivanju dostupnih istraživanja zaključuje se da biotehnološki proizvodi i primjene kao što su dodaci hrani smanjuju proizvodnju metana kao rezultat obuzdavanja upotrebe vodika bakterija koje proizvode metan povećanjem proizvodnje propionske kiseline u buragu.

Ključne riječi:proizvodnja metana, metanogeneza, fermentacija u buragu, biotehnološke metode